Determine the level of stringency that will impact significantly on cross-reactivity. Report and describe any sequence(s)/loci/chromosome region(s) that are shown to cross-hybridize at stringency lower than what is recommended. Photographs of cross hybridization loci would be useful.

Demonstrate that the device can reliably stay within tolerances for the recommended stringency conditions.

When multiple probes are used in an assay, evaluate the stringency requirements for each probe utilized and characterize any difference in the optimal level of stringency among the probes.

D. LOCALIZATION OF TARGET

Document the localization (unique map position) of the target of interest. This may be accomplished at the chromosome band level by sequential testing, e.g., ISH analysis followed by banding. More precise localization may require other genome mapping approaches. The precise location should be reported using standard chromosome banding/gene mapping nomenclature. It may be useful in some cases to search appropriate genome data bases to rule out sequence homology to other regions of the genome.

E. INTERFERENCES

Describe and test for any potentially interfering substances. Provide data to demonstrate that potentially interfering substances encountered in the specimen or assay procedure do not affect assay results.

Address background noise/non-specific binding. Establish that background staining of the slide surface and specimen regions does not interfere with the ability to detect specific signal and does not lead to false detection of specific signal.

F. PRECISION/REPEATABILITY AND REPRODUCIBILITY STUDIES

Design studies that will challenge the device's ability to provide reproducible results under both homogeneous and heterogeneous conditions across a wide range of values, especially within each medically relevant range of values and representative of the intended use.

Studies should be designed with an appropriate number of levels and repetitions to provide statistically meaningful analyses. For quantitative assays, the National Committee for Clinical Laboratory Standards (NCCLS) EP5-T2

recommends an analysis of variance experiment that permits separate estimation of between-day, between-run, and within-day standard deviation (SD) as well as within run and total SDs. Alternative, equally rigorous, protocols published in peer reviewed journals or texts are acceptable.

1. Provide data for inter-observer and/or inter-laboratory, inter-assay, inter-lot (for PMAs), and, when appropriate, intra-assay variability.

2. Study a minimum of 5 to 10 known, simulated and/or actual clinical, specimens with representative values that span relevant levels of signal detection, including values near any medically relevant cut-off. The frequency of repeated testing (e.g., times per specimen, day, week, etc.) should reflect normal conditions for use.

4. Provide data generated at a minimum of three study sites to assess site-to-site/inter observer variability. If labeling allows for replacement of kit components and protocols for sample processing, hybridization, and probe detection, data must be generated by a sufficient number of laboratories to account for additional inter-laboratory variation due to these variables.

5. Lot-to-lot variability (for PMAs):

Provide data using three different lots. Describe the probability sampling plan used in selecting the sample lots.

G. STABILITY DATA

For PMA submissions, stability data must be submitted for all kit reagents as well as the finished kit to ensure the reliability of the device's test results over the shelf-life of the product. These studies should be conducted on unreconstituted and reconstituted reagents from at least three different lots for long-term stability. Data should also be available if requested for 510(k) submissions.

1. Real time studies from three different manufactured lots are required for all PMA submissions. Certain 510(k) submissions may also require these studies depending on the issues of safety and effectiveness of the device.

2. Accelerated stability studies are acceptable as interim data.

3. Shipping and storage conditions: Include data to demonstrate

that all reagents are stable under variable and extreme shipping conditions (e.g., time and temperatures) that may be encountered or state why the device would not be affected by such variable conditions.

V. DIAGNOSTIC PERFORMANCE PROPERTIES

Diagnostic performance refers to the ability of the device to correctly measure or predict the diagnostic endpoint of interest, e.g., clinical outcome (phenotype) and/or genetic status (genotype), whereas, analytic performance deals with the devices ability to provide consistent and reliable results in measuring the target of interest. Ideally, the performance of the device for both diagnostic endpoints should be established (i.e., phenotype and genotype); especially for novel and unproven applications.

At a minimum, the parameters described below, i.e., expected values, diagnostic/relative sensitivity and specificity, and predictive values, should be validated for the device's intended use in the specified target population(s) with appropriate studies, as applicable. In cases where additional clinical/diagnostic claims are made, e.g., predictive, prognostic, etc., other types of analyses may be required.

A. SELECTING AND ESTABLISHING DIAGNOSTIC ENDPOINT(s)

Diagnostic endpoints should be selected that permit appropriate statistical methods of analysis to assess the diagnostic performance properties of the device for its intended use and target population. The diagnostic endpoint (e.g., relevant phenotype/genotype) should be established for each subject entered into the studies using the best diagnostic criteria available, e.g., accepted clinical assessment criteria, laboratory testing, genetic linkage studies, family and medical history, etc., as appropriate.

In situations where alternative molecular methods are available which are well documented in the literature, the FDA may, on a case by case basis, accept such data to define the diagnostic endpoint when used in conjunction with other accepted methods of evaluation.

B. EXPECTED VALUES

Establish expected values (reference range, cut-off values) for the target population and for each reference population, i.e., for each relevant state of nature (including mosaicism/minimum residual disease, if applicable), as obtained by the device from studies of appropriate populations. Evaluate specimens from appropriately selected individuals reflective of the intended target population. Document the diagnostic criteria used for characterizing the study subjects.

The following should be addressed as appropriate:

1. Percentage distribution of signals per cell:

For interphase analysis, establish the distribution of cells with the relevant number of signals/cell, e.g., 0, 1, 2, 3, 4, etc. (see III.A.8.), for each relevant cell population, e.g., diploid and aneuploid, including mosaicism, if appropriate. The category(ies) of the signal distribution may be grouped together, when appropriate, to provide the most informative method of analysis.

2. Assay cut-off:

When applicable, determine the assay cut-off for distinguishing between relevant clinical or genetic states (e.g., affected vs. carrier vs. normal). For evaluation of mosaicism or detection of minimum residual disease, define the criteria or cut-off which differentiates between normal vs. abnormal [e.g., true mosaicism (usually monosomy or trisomy), etc. For example, when interphase analysis is intended to detect minimum residual disease associated with a trisomic cell line, establish the percentage of tri-signaled cells that will distinguish a predetermined level of mosaicism. This might be accomplished with well characterized simulated specimens in which cells from subjects with one state of nature are mixed with others at a known level and subsequently quantitated for comparability. Document the criteria for characterizing specimens utilized for simulation.

In some cases, it may be appropriate to validate the cut-off with an independent data set other than that which was used to determine the analytic performance parameters of the device or by an equivalent statistical procedure such as bootstrapping or other resampling method.

Define the statistical method used to establish the cut-off and what properties the cut-off was designed to have, such as maximizing the specificity and sensitivity for a particular use in the target population. ROC curve analysis is commonly used to yield a measure of how good the cut-off is in a particular application. Present ROC curve analysis of cut-off selection as appropriate.

3. Prevalence of disorder/target:

Provide the expected prevalence/frequency of the disorder/target for the relevant intended use population(s) or explain why this is not feasible.

Use appropriate subject selection criteria when assessing the distribution and prevalence within the target population. For example, consider the appropriateness of using specimens from unrelated vs. related individuals. Characterize any known variability in expected prevalence between relevant subpopulations and other variables as appropriate. State the reason(s) for this variability, e.g., geographic, racial, ethnic, etc.

4. Population-based location specificity:

Demonstrate whether the genetic target of interest is limited to a unique [physical] map position within the population. Some target sequences have been documented to lack location specificity (i.e., variability in map position (chromosome/region location) of the target sequence). For example, the target for probe D15Z1 is usually on chromosome 15. However, in some individuals, this sequence is on chromosome 13. Note that the concept of location specificity at the population level is not the same as a probe's analytic specificity for the target of interest. Location specificity refers to the chromosome/region/locus where the target of interest may normally reside whereas analytic specificity (or lack of specificity) relates to the probe of interest hybridizing to the target vs. non-target as a function of stringency conditions and sequence homology to non-target.

For all devices, perform appropriate studies and/or provide evidence from appropriate literature references to characterize any lack of location specificity. A sufficient number of unrelated individuals (or evidence from the published literature) should be evaluated to rule out variability in target location at the level of a true polymorphism (i.e., target found at an alternate location in as few as two percent of the population). Localization can be confirmed by sequential testing, e.g., ISH analysis followed by banding.

The number of subjects that must be studied to characterize this property should be determined on a case-by-case basis for the class of probes being evaluated. Probes to the alpha-satellite region generally require a larger population sampling to determine population variation. Alternately, for probes to single copy sequences that are known or not expected to be polymorphic, fewer individuals may need to be tested. Describe the expected variability at the population level or justify why this is not relevant.

C. DIAGNOSTIC SENSITIVITY AND SPECIFICITY

Diagnostic sensitivity and specificity are two key performance parameters important for evaluation of a device's safety and effectiveness. These parameters measure the diagnostic accuracy of the device.

Well designed studies are necessary to establish a device's diagnostic performance. When accepted diagnostic criteria exist for a specific disorder, the diagnostic sensitivity and specificity are determined by comparing the device's test results to the true, known, clinical/genetic diagnosis (see Figure 3).

Disorders with low prevalence or other unusual phenomenon responsible to expression of the disorder, e.g., delayed onset, variable expressivity, etc., present unique study design problems. For very rare disorders, e.g., Miller-Dieker syndrome, it may be difficult to obtain a sufficient number of cases. Since many such cases arise from unbalanced segregation of a parental chromosome abnormality, unaffected carriers of the balanced rearrangement may provide useful information.

Validation of devices intended for prenatal diagnosis in which fetal test results indicate the presence/absence of disease, will depend on follow-up of prenatal test results or on test results in living subjects. For claims involving prenatal diagnosis for detection of visible chromosome abnormalities, test a series of pregnancies in parallel with standard cytogenetics or other accepted technique(s) to compare the diagnostic performance of the device to the prevailing "gold standard". Confirm the provisional diagnosis by follow-up evaluation of newborns or abortus tissue.

Calculate the test's clinical/diagnostic sensitivity and specificity as follows and determine the confidence limits for the estimates (see Fig. 3). Confidence intervals should be calculated using the binomial probability distribution unless the sample size is sufficiently large to justify using the normal approximation to the binomial.

1. Diagnostic sensitivity:

For ISH testing, "disease positive" may be interpreted as the presence of a particular genetic state of nature; whether the state of interest is the genotype (e.g., heterozygous state vs. homozygous state) or phenotype (clinical features). Determine the following parameters, when appropriate, and state the confidence interval for the estimates or justify why this is not feasible:

a. Determine the ability of the device under study to give a positive result for subjects having the disease, relevant target

sequence or correct number of targets, or state of nature of interest (e.g., genotype or clinical phenotype) [i.e., calculate the probability (P) that the test is positive (T+), given that the subject being tested is disease/state of interest positive (D+), i.e., P(T+|D+)].

b. Define the proportion of all cases with a particular state of interest not detectable by interphase ISH. Calculation of this measure must take into account multiple mechanisms responsible for the state of interest, such as a particular clinical phenotype. For example, if a device is intended to screen for aneuploidy, prenatally, using probes for chromosomes 13, 18, 21, X, and Y, what is the frequency of other chromosome abnormalities which would not be detected by the device. The Down syndrome (trisomy 21) is caused by a primary trisomy, unbalanced translocation, or mosaicism for chromosome 21. When applicable and for prenatal testing, the calculation should be based on current frequency data of numerical and structural abnormalities that would go undetected with use of the device at any stage of gestation at which it is done. If the calculations vary substantially with clinical indications for patient testing, data will need to be presented by indication.

2. Diagnostic Specificity:

For subjects who do not have the disease, relevant target sequence, correct number of targets, or the state of nature of interest, e.g., genotype or clinical phenotype, (D-), determine the proportion who test negative with the device (T-) and report the confidence intervals for the proportion, P(T-|D-).

D. METHODS COMPARISON FOR 510(k)

In some cases, it may be appropriate to compare the performance of the new device to the performance of another method if the method is recognized as a "gold standard". In instances where standard cytogenetic analysis is well documented to reliably characterize a desired endpoint, e.g., numeric or visible structural chromosome abnormalities, and for which the clinical correlations are well documented, the performance of standard cytogenetic analysis may be used to characterize the performance of the new device. When directly comparing a new device to a comparison method, only "relative" performance, e.g., "relative" diagnostic sensitivity and specificity (see Fig. 3), can be determined.

Direct comparison of a new device to a comparison method which is less than optimal is discouraged. This approach is problematic in that calculation of relative sensitivity and specificity will give a biased impression of the true performance of the new device since the true status, whether the test results of the two methods are in agreement or discrepant, is not certain. Such comparisons frequently generate a substantial number of discrepant results. Attempts to resolve discrepant results for purposes of calculating performance parameters will further bias the impression of a device's performance. When the comparison method is less than optimal and its true diagnostic performance is not well established, the diagnostic sensitivity and specificity for both the new device and standard cytogenetics should be determined by comparing the performance of each method, independently, to the desired diagnostic endpoint. Statistical tests may then be applied to determine how each device performs in comparison to the "gold standard". The performance properties of the new device should be as good as existing methods for a substantial equivalence determination to be made.

E. PREDICTIVE VALUES

Positive predictive values (PV+) and negative predictive values (PV-) are key to evaluating the effectiveness of a test in the target population(s), especially for tests intended for broad-based population screening. The predictive values are a function of the prevalence of the state of interest in the target population and the diagnostic sensitivity and specificity of the test. For the predictive values to be adequately estimated, the sensitivity, specificity, and prevalence must be estimated with sufficient accuracy.

Predictive values are difficult to calculate accurately for rare disorders of unknown prevalence. Further compounding this problem is the variable expressivity of certain disorders such that the frequency of a positive test result is highest in those with the most classical phenotypic presentation and becomes lower as the number of phenotypic features of a syndrome for which the patient presents decreases.

Since the test will be performed by various users in demographically different populations, each with potentially different prevalence, the manufacturer should provide predictive values together with their accuracy on a wide range of population prevalence. Prevalence data from published literature may be acceptable in some cases for use in estimating predictive values for different prevalences, under 510(k), provided extrapolation of such data can be justified.

Provide the positive and negative predictive values for the device, when applicable. The predictive values are determined as follows:

$$PV(+) = \frac{S_1 P}{S_1 P + (1 - S_2)(1 - P)}$$

$$PV(-) = \frac{S_2(1 - P)}{(1 - S_{1)}P + S2)(1 - P)}$$

where:

T = test result, D = disease/state of interest, P = prevalence of disease/state of interest (i.e., frequency of disease/state of interest in the target population), S_1 = diagnostic sensitivity and, S_2 = diagnostic specificity.

Predictive values as stated in this section represent population values and do not necessarily represent the probability that a given individual will have the genetic/diagnostic state, given a particular test outcome.

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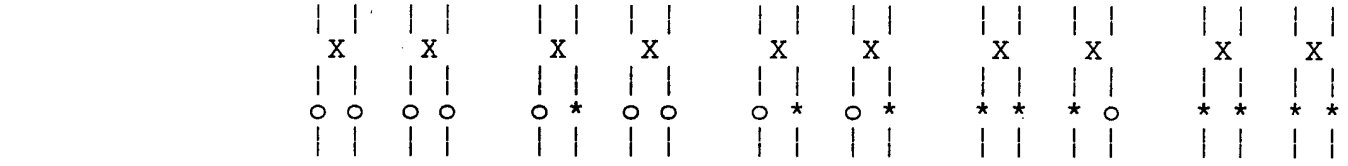
Figure 1. SIGNAL SCORING FOR NONCENTROMERIC PROBES IN METAPHASE CELLS

Chromatid scoring, per chromosome and per chromosome pair:



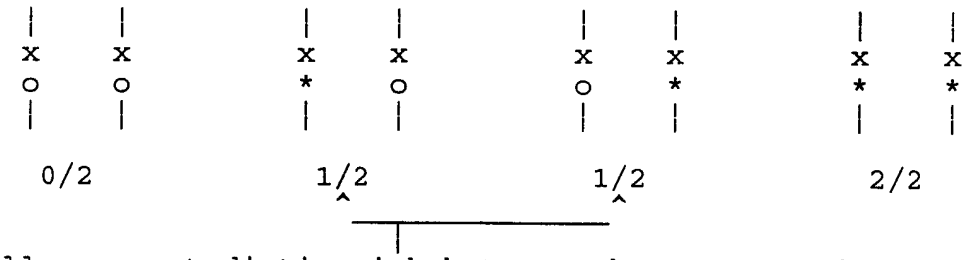
Per chromatid:	0/2	0/2	1/2	0/2	1/2	1/2	2/2	1/2	2/2	2/2
Per pair:	0/4		1/4		2/4		3/4		4/4	

o = no signal at target site; * = signal at target site; X, x = centromere

NOTE: Describe and quantify all observed recurrent patterns not represented in this diagram, e.g., cases of aneusomic chromosomes which contain deleted or amplified regions or signals on other chromosomes which may periodically stain.

Figure 2. SIGNAL SCORING FOR CENTROMERIC PROBES IN METAPHASE CELLS

Chromosome scoring, per cell (chromosome pairs with target sequence)



Usually can not distinguish between chromosomes of a pair unless there are heteromorphisms of satellite DNA, e.g., chromosomes 13 to 15 and 21 and 22.

NOTE: Describe and quantify all other observed recurrent patterns not represented in this diagram, e.g., chromatid separation or dicentric chromosomes.

Figure 3. CALCULATING THE DIAGNOSTIC PERFORMANCE PROPERTIES

NEW DEVICE TEST RESULTS	DIAGNOSTIC STATUS (+)	(-)	
Test (+)	a	b	a + b
Test (-)	c	d	c + d
	a + c	b + d	a + b + c + d

KEY: assay/diagnosis

a =	+/+	true positive
b =	+/-	false positive
c =	-/+	false negative
d =	-/-	true negative

Diagnostic sensitivity = a/(a + c), and

Diagnostic specificity = b/(b + d)

PV(+) = a/(a + b)*

PV(-) = d/(c + d)*

*Calculation of predictive values using this approach are meaningful only if the new device is compared to true diagnostic status and if "disease" prevalence in the study population is representative of disease prevalence in the target population. See definitions in section V.E. above.

NOTE: The same approach may be used for comparing a new device to a "gold standard" method. Diagnostic status in the table would be replaced by definitive method test results. Such a comparison will result in an estimate of "relative sensitivity" and "relative specificity".

Figure 4. ISH PROBE APPLICATIONS

Centromeric Repeat Probes:

* Metaphase and interphase analysis to identify specific chromosomes and chromosome regions.

Site Specific Probes:

* Microdeletion and unique sequences in metaphase and interphase.
* Chromosome enumeration in metaphase and interphase.
* Duplication (including amplification) or deletion of corresponding chromosome regions, e.g., Charco-Marie-Tooth type 1A (duplication of 17p11.2-12).

Whole Chromosome Paints

* Characterize chromosome rearrangements.
* Assignment of breakpoints.
* Determine the origin of marker chromosomes/extra chromosomal material.
* Determine chromosome number in metaphase.

The utility for interphase use is limited.

Telomere Probes

* Characterize chromosome translocations and/or marker chromosomes in metaphase, especially when used in conjunction with the related centromere probe.

The utility for interphase use is limited.

APPENDIX A

BACKGROUND FOR *IN VITRO* DIAGNOSTIC DEVICES THAT UTILIZE CYTOGENETIC *IN SITU* HYBRIDIZATION TECHNOLOGY FOR THE DETECTION OF HUMAN GENETIC MUTATIONS (CONSTITUTIONAL AND SOMATIC)

Historical:

The clinical relevance of chromosome abnormalities was first demonstrated with the identification of trisomy 21 in the Down syndrome in 1959. With the advent of improved chromosome banding techniques, cytogenetic analysis became well established in clinical practice for detection of constitutional and somatic chromosome abnormalities of both number and structure in metaphase cells. Constitutional abnormalities may be either inherited or sporadic. Chromosome abnormalities are present in up to 50% of spontaneous abortions and in 0.6% of all live births; most are sporadic mutations.[1] Abnormalities of primary interest in prenatal testing are numeric constitutional abnormalities, although post zygotic events leading to mosaicism are significant. The most commonly detected chromosome abnormalities in the newborn are numeric, e.g., trisomy 21, sex chromosome aneuploidies, etc. In addition, structural abnormalities are significant and include both balanced and unbalanced rearrangements.

Recurrent, nonrandom cytogenetic abnormalities (both number and structure) are well documented to be associated with specific tumors, and include both inherited and sporadic constitutional mutations and acquired somatic mutations. Cytogenetic abnormalities associated with human cancer can be used as markers to characterize and aid in the diagnosis of certain types of cancer for purposes of patient management.[2] Detection of such disorders has relevance to both hematological malignancies and solid tumors.

High-resolution banding techniques make possible the visualization of structural alterations of single chromosome bands. These techniques do not, however, permit resolution at the gene or submicroscopic level. Cytogenetic analysis is limited to cycling metaphase cells and usually requires culturing the specimen to obtain sufficient numbers of metaphase cells for analysis. Culturing may result in an unintended selection of the cell populations and result in a cell type distribution different from that found in the original specimen. These and certain other limitations of traditional cytogenetic techniques may be circumvented with the introduction of ISH applications for clinical use.

ISH Technology

Nucleic acid hybridization (either DNA or RNA) applied to intact cells is referred to as *in situ* hybridization (ISH). ISH was developed in the late 1960s and was applied to localizing specific nucleic acid sequences in cytologic preparations.[3,4] ISH makes possible the localization within a specific nucleic acid sequence to a specific cell, cell structure, or organelle.

Nucleic acid hybridization involves the pairing of two complementary (anti-parallel) single strands of nucleic acid resulting in a double stranded (duplex) molecule. Base pairing is based on the formation of hydrogen bonds between complementary base pairs. This makes possible DNA:DNA, DNA:RNA, and RNA:RNA duplexes. Therefore, if a nucleic acid probe has nucleotide sequences complementary to the target, the probe will hybridize (bind) to the target under appropriate conditions. Depending on the target nucleic acid selected, the probes may be used to detect specific chromosomes, or specific sites.

Probes used for ISH may be labeled either isotopically or nonisotopically by incorporating modified nucleotides. The modified nucleotides contain appropriate isotopic tags (e.g., ^{32}P, etc.) or nonisotopic fluorophores linked to biotin, digoxigenin, etc. Many different radioactive and nonradioactive probes have been described for use with ISH. Hybridized labels may be visualized directly or indirectly. Indirect detection requires use of reporter molecules (e.g., fluorescently tagged avidin or antidigoxigenin antibody for detection of biotin and digoxigenin, respectively). Fluorescent reporter molecules are commonly used for probe detection in cytogenetic ISH analysis.

ISH test methodology involves the following basic steps: 1) collection and preparation of sample, 2) denaturation of the sample (target) nucleic acid, 3) hybridization of labeled probe to complementary, denatured target, 4) washing away excess probe, and 5) detection of the specifically bound residual probe.

Key factors that affect the analytic performance of the assay include: degree of homology between the probe and target sequences, probe construct/labeling and length, specimen pretreatment, hybridization conditions, and sensitivity of the signal detection method. Numerous protocols are available to optimize the hybridization reactions.[5,6]

Hybridization conditions which control duplex association/dissociation are referred to as stringency. The degree of stringency is regulated by varying the conditions of the hybridization reaction, particularly, temperature and concentrations of salt (ionic strength) and organic solvents such as formamide. Low stringency conditions may result in cross-hybridization to regions in the genome that are different from the target sequence but have some homology to the target. High stringency may be attained with high temperature, low salt, or high formamide concentrations; or a combination of

all three. Duplexes formed by two strands that have a high degree of base homology (pairing) will better withstand high stringency washing than duplexes with less homology.

Strict adherence to procedure parameters that critically affect the assay performance characteristics is essential to assure the safe and effective use of ISH devices for IVD use.

ISH Applications:

ISH has been used extensively for purposes of gene mapping. The use of ISH for cytogenetic detection of constitutional and acquired mutations is rapidly moving from research and investigational use to diagnostic use. Diagnostic use of ISH will impact significantly on diagnostic and patient management decisions. Selection of appropriate hybridization probes is dependent on clinical and diagnostic indications. In some cases, use of multiple probes may be required to delineate/identify a particular abnormality.

Numerous probes are being developed to facilitate the characterization and detection of chromosomal abnormalities that are not possible to do by standard cytogenetic methods. There are several major categories of probe products with potentially significant clinical relevance: 1) chromosome "painting" probes directed at whole chromosomes or chromosome regions, 2) centromeric probes directed at repetitive DNA sequences, e.g., alpha, beta and other satellite, 3) site-specific probes, and telomeric probes. In addition, total genomic DNA may have some limited applications (e.g., detection of polyploidy).

Whole chromosome "paints" may be valuable for characterizing complex translocations, determining the origin of marker chromosomes, derivation of de novo material, assignment of breakpoints, etc., in metaphase chromosomes. The utility for interphase use is limited but may be useful, in some cases, for aneuploidy detection.

Centromeric probes are aimed, primarily, at identifying specific chromosomes and chromosome regions in metaphase and interphase cells. They are useful for detecting aneuploidies in interphase cells although some may have limited use in characterizing known chromosomal abnormalities.

Site-specific probes are intended to detect alterations in the structure or number of copies of the target sequence. These probes may be directed at detecting abnormalities at a unique chromosomal locus including single and contiguous gene mutations such as deletions (including microdeletions), unique sequences, translocation breakpoints, inversions, duplication (e.g., Charco-Marie-Tooth type 1A, duplication of 17p11.2-12) including amplification (e.g., double minutes and

heterogeneous staining region), etc. In addition, they may be useful for chromosome enumeration. These probes are generally useful for both metaphase and interphase applications.

Telomeric probes are useful for characterizing chromosome translocations and/or marker chromosomes in metaphase, especially when used in conjunction with related centromere probes. The utility for interphase use is limited.

There are a number of new ISH applications and methodologies under research and development. A relatively new technology, Comparative Genomic Hybridization, is aimed at identifying regions of the genome with increased gene copy number or deletions. Other applications are being developed that include quantitative hybridization, digital imaging and automation (flow cytometry), PCR-*in situ* hybridization, use of simple repeats $(CAC)^5$ and M13 minisatellite tandem repeat sequence probes produce an R-band like banding pattern, etc.[7,8,9,10,]

Advantages/Limitations of ISH

The use of ISH for detection of cytogenetic abnormalities has certain advantages and limitations. ISH may be sensitive for detecting submicroscopic lesions in metaphase chromosomes beyond the resolution of standard banding techniques (e.g., certain specific microdeletions, cryptic translocations, etc.). Interphase ISH applications for detection of acquired abnormalities in somatic cells may have potential benefit since it does not require use of cultured or cycling cells. This can eliminate the unintended selection that occurs when one cultures cells from patients with neoplastic diseases with low mitotic indices. ISH permits analysis of thousands of cells vs. only 30 to 100 cells in the usual cytogenetic analysis.

Limitations of the ISH applications are significant, especially for interphase applications. These include but are not limited to the following: 1) risk for false positives resulting from cross-hybridization to other sequences and as a consequence of background noise; 2) risk for false negatives due to alternate genetic mechanisms responsible for a given disorder or reduced analytic sensitivity; 3) inability to distinguish real mosaicism at low levels due to variable hybridization in cell populations; 4) interpretational difficulty associated with detection of low levels of mosaicism; and 5) lack of chromosome specificity of certain probes.[11,12] In addition, interphase analysis fails to provide cytogenetic information comparable to that of standard cytogenetic analysis in which the entire chromosome complement of a cell is examined. ISH is directed at a single abnormality. Therefore, interphase ISH analysis, as a stand alone test, may be less informative.

The clinical and interpretive concerns about interphase ISH for detection of inherited and sporadic constitutional abnormalities (particularly for prenatal) will be similar. The

potential for reduced clinical/diagnostic sensitivity and specificity of interphase analysis as a stand alone raises significant S & E concerns different from standard cytogenetics when applied to detection of constitutional abnormalities, whether prenatally or postnatally. Therefore, any claims for interphase ISH as a "stand alone" test will require careful risk/benefit analysis to demonstrate the S & E of the device prior to the FDA approval/clearance for marketing.

Unique Concerns Raised by Genetic Disease Testing:

Detection of genetic disorders with ISH raises the same unique issues of safety and effectiveness as genetic testing by other methods. False positive or false negative test results may lead to an incorrect clinical or genetic diagnosis. Postnatally, this may influence the course of medical treatment for the affected individual. A erroneous diagnosis, prenatally, may result in inappropriate actions being taken or the woman may not be provided with the information necessary on which to base an informed decision regarding her options.

Testing for constitutional disorders frequently has relevance not only to the subject being tested but also to other family members. Therefore, an incorrect test result may lead to an incorrect interpretation of recurrence risks not only for the subject being tested but also for other at risk family members.

Genetic testing is also unique because of the probabilistic nature of the interpretation of genetic tests and the requirements of knowledge of the genetic basis and mechanisms responsible for the particular disorder. This knowledge includes, but is not limited to the following: mode of transmission, dominant/recessive and autosomal/X-linked; interpretation of family and medical history; frequency of the mutation in the target population; geographic/racial/ethnic distribution of the disorder; alternate mechanisms responsible for the clinical phenotype, e.g., trisomy 21 exists as a primary (numeric), structural or mosaic abnormality; polymorphic nature of the locus in question; allelic diversity (multiple allelic mutations responsible for the disorder); genetic heterogeneity (multiple genes responsible for a given clinical phenotype); chromosome specificity; performance properties of the assay; and knowledge about the application of probability theory.

Because of the complexity of testing and interpretation of test results, genetic IVDs may be subject to special requirements when necessary to assure the safe and effective use of the device. The FDA will require appropriate labeling and provisions that reflect the standard of care under existing laws, regulations, and policies.

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